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1 Diagnostic Biomarkers in Women With Suspected Preeclampsia in a Prospective

2 Multicenter Study

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51 *report any potential conflicts of interest.*

52 **Short title: Diagnostic markers in suspected preeclampsia**

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54

55 **Précis:** In women with suspected preterm preeclampsia, a single angiogenesis-related

56 biomarker is a useful diagnostic test to determine preeclampsia that requires delivery within

57 14 days.

58

59

60 **Abstract**

61 **Objective:** To evaluate 47 biomarkers (selected from the current medical literature), in
62 isolation or in combination with placental growth factor (PIGF), to determine the need for
63 delivery within 14 days, in women presenting with suspected preterm preeclampsia.

64

65 **Methods:** In a prospective, multicentre observational study, 47 biomarkers were measured
66 in 423 women presenting with suspected preterm preeclampsia (in two prespecified groups:
67 Group 1 at <35 weeks of gestation and Group 2 presenting between 35⁺⁰ and 36⁺⁶ weeks of
68 gestation), to evaluate their ability to determine the primary endpoint: preeclampsia
69 requiring delivery within 14 days. Using factor analysis and stepwise logistic regression, we
70 sought one or more additional biomarkers for optimal determination of the primary
71 endpoint.

72

73 **Results:** In women presenting <35 weeks of gestation (n=286), the best-performing
74 combination of PIGF, podocalyxin, endoglin, procalcitonin (receiver operating curve (ROC)
75 area 0.90; 95% CI 0.86 to 0.93) was not statistically better than PIGF alone (ROC 0.87; 95% CI
76 0.83 to 0.92; p=0.43) for preeclampsia requiring delivery within 14 days. Two other single
77 markers had test performance that was not significantly different to PIGF (soluble fms-like
78 tyrosine kinase-1 [sflt-1] ROC 0.83; 95% CI 0.78 - 0.88; endoglin ROC 0.83; 95% CI 0.79 -
79 0.88). Similar findings were found in women presenting between 35⁺⁰ and 36⁺⁶ weeks of
80 gestation (n=137): ROC for PIGF alone 0.75 (95%CI 0.67 to 0.83); ROC for PIGF, cystatin,
81 pregnancy-associated plasma protein A (PAPP-A) in combination 0.81 (95% CI 0.74 to 0.88;
82 p=0.40).

83

84 **Conclusions:** This study supports the growing body of evidence that a single angiogenesis-
85 related biomarker (PlGF, sFlt-1 or endoglin) alone represents a useful diagnostic test for
86 women presenting with suspected preterm preeclampsia.

87

88

89 **Introduction**

90 Preeclampsia is a common disorder affecting between 5-7% of all pregnancies.(1) It remains
91 a major contributor to maternal mortality(1) and accounts for a substantial proportion of
92 low birthweight infants and iatrogenic preterm delivery.(2) Prevalence and morbidity has
93 remained unchanged over the last decade highlighting the need to improve diagnostic(3, 4)
94 and prognostic(5) testing facilitating appropriate resource allocation. Preeclampsia is unique
95 to pregnancy and is characterised by poor placentation(6) and abnormal inflammatory and
96 vascular responses(7) resulting in multi-organ dysfunction.

97 Presenting symptoms of preeclampsia are often subjective and non-specific with
98 clinical findings based on features of advanced disease or markers of end organ involvement.
99 High blood pressure and urinary protein excretion are typically used to diagnose the disease
100 but both are secondary features of a primary placental problem and subject to
101 measurement error and poor test accuracy.(8) It is currently difficult to distinguish
102 preeclampsia of a severity that requires early delivery from other less serious phenotypes.(9,
103 10) An accurate biomarker (or panel of biomarkers) to enable prognosis of perinatal
104 complications could have substantial impact on management strategies with the aim of
105 minimising adverse maternal and fetal outcomes.

106 The aim of this study was to evaluate a wide panel of 47 candidate biomarkers
107 (including those that are currently widely reported and reflect the heterogeneity of the
108 disease) in women presenting preterm with suspected preeclampsia in order to optimise
109 determination of an important clinical outcome, that of preeclampsia requiring delivery
110 within 14 days.

111

112 Materials and Methods

113 A prospective multicentre cohort study was undertaken between January 2011 and February
114 2012 in seven consultant-led maternity units in the United Kingdom and Ireland.(4) Women
115 were eligible for the study if they had been referred or presented with suspected
116 preeclampsia (i.e. signs or symptoms of preeclampsia), were 20⁺⁰ to 36⁺⁶ weeks of gestation
117 with a singleton or twin pregnancy and were aged ≥ 16 years. Women with confirmed
118 preeclampsia (or with any adverse outcome already present) were not eligible. We
119 undertook a planned analysis reported here on two groups of women: Group 1: presenting
120 prior to 35 weeks of gestation, and Group 2: presenting between 35⁺⁰ and 36⁺⁶ weeks of
121 gestation. These gestational age groupings were pre-specified, based on known differences
122 in pathophysiological pathways associated with preterm pre-eclampsia and our prior
123 knowledge of gestational changes of biomarker concentrations related to these pathways.
124 Written informed consent was obtained and baseline demographic and pregnancy-specific
125 information, including blood pressure readings, were entered onto the study database.
126 Blood pressure was taken according to unit guidelines. Blood samples were drawn into
127 ethylenediamine tetra-acetic acid, with consent, at the time of enrolment. The samples were
128 labelled, transported to the laboratory and the plasma was stored until analysis at -80°C.
129 Pregnancy outcomes were determined by case note review with independent adjudication
130 (masked to all biomarker concentrations) for final maternal diagnosis. All hypertensive
131 disorders of pregnancy were defined according to the American College of Obstetricians and
132 Gynaecologists practice bulletin in use at the time of the study.(11) Independent
133 adjudication was undertaken by two senior physicians, masked to biomarker measurements,
134 requiring documentation of end points required to fulfil the diagnostic criteria; disagreement
135 was resolved by a third adjudicator. The predefined adverse maternal outcomes had been

identified for a previous study in preeclampsia by iterative Delphi consensus(10) and have been described in detail elsewhere.(4) All sites managed women (including decision for delivery) in line with the Hypertension in Pregnancy recommendations from the National Institute for Health and Care Excellence.(12)

An initial panel of biomarkers was selected based on either *a priori* knowledge of an association with preeclampsia, a biological role in placentation or a role in cellular mechanisms involved in the pathogenesis of preeclampsia e.g., angiogenesis, inflammation, coagulation. The full list of 47 biomarkers, measured with 57 assays (where potentially biologically important assays of different epitope specificity were available) was generated following a review of the literature, appraisal of selected bibliographies and consultation with medical experts (Appendix 1, available online at <http://links.lww.com/xxx>).

Plasma samples were tested for Placental Growth Factor (PIGF) using the Triage PIGF Test by trained laboratory staff at the study site where the sample was taken (as previously published). Samples were labelled, and transported to the laboratory where they were spun at 3000 rotations per minute for 10 minutes. The additional 56 biomarker assays were analysed in a central laboratory facility (Alere, San Diego, CA) and full details of assay methods given in Appendix 2, <http://links.lww.com/xxx> and Appendix 3, <http://links.lww.com/xxx>. All participants had delivered and pregnancy outcomes recorded before biomarker concentrations were analysed and revealed and all laboratory staff were masked to clinical outcomes.

Standard distributional checks showed high levels of skewness for all 57 assays, consistent with underlying log normal distributions. Logged values of these biomarkers were therefore used. Before considering the pregnancy outcomes, statistical factor analysis of biomarker data was undertaken, reducing the 47 biomarkers into a smaller group of factors. Factor

analysis sorted the biomarkers into a small number of highly correlated groups, without reference to outcome, containing the majority of the information in the full dataset.(13)

Factor summary scores were then calculated for all women. Consideration of scree plots and Eigen-values (> two) identified the most important factors for further analysis.(14) These factors were rotated (orthogonal varimax method) so that each factor related strongly (correlation >0.6) to a small number of biomarkers only (factor analysis is displayed in Appendix 4, <http://links.lww.com/xxx>). Significant factors (and their biomarkers) were identified for further investigation (Appendix 5, <http://links.lww.com/xxx>). For the multiple logistic regression model, the principal outcome was preeclampsia requiring delivery within 14 days (pre-specified by consensus of clinical investigators). Stepwise logistic regression was used to determine which biomarkers or factors appeared to provide additional information beyond that derived from PIGF and prediction scores were extracted for the best combinations. A comparison of Receiver Operating Curves (ROC) areas of individual biomarkers and combinations was made to see if any of the additional information was both consistent and large enough to be clinically useful. Significance was assessed through use of a non-parametric test which allowed for non-independence of observations on the same participant, with Bonferroni correction for multiple testing.(15)

Some biomarkers, with high uniqueness scores, were not strongly associated with any factor. To investigate whether any of these biomarkers had diagnostic power in addition to that provided by PIGF and biomarkers identified earlier, stepwise logistic regression was undertaken. To avoid excluding a biomarker that may be of potential value, it had to pass a series of tests, so that the chance of a false positive was greatly reduced (rather than using a standard multiple-testing correction to p-values, such as Bonferroni). The biomarker had to be a component of a significant factor, a significant predictor in logistic regression both

alone and after allowing for PlGF and have a ROC area for the combined score significantly greater than PlGF alone. For biomarkers with a substantial proportion of measurements outside the limits of detection, we used a non-parametric test (ROC area) to determine whether the biomarkers had useful predictive power. Where the biomarker measurement (whether due to censoring or lack of predictive ability) was non-informative, it was excluded from further analysis.

Statistical analysis was carried out in the statistical package Stata (version 11.2), College Station Texas, USA. Clinical variables and outcomes were compared using a Wilcoxon rank-sum non-parametric test. The pre-specified sample size was calculated for accurate estimation of the sensitivity (within 10%) and specificity (within 6%) of a biomarker, assumed a sensitivity of 0.90, specificity 0.90, and 95% confidence intervals (2-tailed), for determining the primary endpoint; this required 62 preeclampsia cases and 150 women not meeting the primary endpoint. The study is reported in accordance with STROBE guidelines (). The study was approved by East London Research Ethics Committee (ref. 10/H0701/117). Participants gave informed consent and the study followed institutional guidelines.

Results

Four hundred twenty three women with enrolment samples and outcome data available were recruited to the study in seven centres across the UK and Ireland between January 2011 and February 2012, 286 women in Group 1 (presenting at 20⁺⁰ to 34⁺⁶ weeks of gestation) and 137 women in Group 2 (presenting at 35⁺⁰ to 36⁺⁶ weeks of gestation) (Figure 1).

For the 286 women who were enrolled prior to 35⁺⁰ weeks of gestation, characteristics of the study population at antenatal booking are shown in table 1, subdivided into those that

208 met the primary outcome (pre-eclampsia requiring delivery within 14 days) and all others.

209 Table 2 shows characteristics of delivery and maternal and neonatal outcome. Table 3 shows

210 the test performance for the most promising individual biomarkers, depicted by ROC areas.

211 PlGF had the highest ROC area (0.87) for determining preeclampsia requiring delivery within

212 14 days; the ROC areas for sFlt-1 (0.83) and endoglin (0.83) were not significantly different to

213 that for PlGF. Addition of further biomarkers to PlGF increased the ROC area by a small, non-

214 significant increment only. The highest test performance for preeclampsia requiring delivery

215 within 14 days was found using a combination of PlGF, podocalyxin, soluble endoglin and

216 procalcitonin, with a ROC area of 0.90, not significantly greater than the ROC area for PlGF

217 alone (0.87; $p=0.43$). Appendix 6, <http://links.lww.com/xxx> shows ROC areas for all 47

218 biomarkers analysed and individual median biomarker concentrations in all women sampled

219 are shown in Appendix 7, <http://links.lww.com/xxx>. Sensitivity analysis demonstrated that

220 excluding twin pregnancies altered PlGF test performance by <1%.

221 For women presenting between 35⁺⁰ and 36⁺⁶ weeks of gestation ($n=137$), the characteristics

222 at booking and enrolment are shown in Appendix 8, <http://links.lww.com/xxx> and those for

223 delivery and pregnancy outcomes in Appendix 9, <http://links.lww.com/xxx>. ROC areas and

224 individual median biomarker concentrations for the individual biomarkers are given in

225 Appendix 10, <http://links.lww.com/xxx> and Appendix 11, <http://links.lww.com/xxx>,

226 respectively. The results follow a similar pattern as for women presenting at earlier

227 gestations. The ROC area for PlGF alone (0.75; 95% CI (0.67 to 0.83)) in determining need for

228 delivery for preeclampsia within 14 days was lower than that achieved in earlier gestations

229 and other angiogenesis-related biomarkers were not significantly different to that for PlGF

230 alone. Integration of soluble fms-like tyrosine kinase-1 (sFlt-1) with PlGF (as a ratio)

231 increased the ROC to 0.77 (95% CI 0.69 to 0.84). The combination of PlGF, pregnancy-

232 associated plasma protein A and cystatin yielded the highest ROC area of 0.81 (95% CI (0.74
233 to 0.88) (table 4). Both increments were small and not significant.

234

235 Discussion

236 This prospective multicentre study is a comprehensive direct comparison of diagnostic
237 biomarkers for preeclampsia. The results demonstrate that in women with suspected
238 preeclampsia presenting preterm, use of a single angiogenesis-related biomarker (PlGF, sFlt-
239 1 or endoglin) alone represents a useful diagnostic test for determining preeclampsia
240 requiring delivery within 14 days, a relevant endpoint indicating that a clinician has
241 considered that the risks of adverse outcomes associated with ongoing expectant
242 management are outweighed by the risks of delivery.

243 Suspected hypertensive disorders in pregnancy are the commonest reason for
244 presentation for obstetric assessment in the third trimester of pregnancy. Diagnostic
245 uncertainty is common when women present to obstetric assessment units with one or
246 more signs suggestive of preeclampsia. Women undergo a series of investigations, many of
247 which are poor predictors of the need for delivery or likely adverse outcome. In practice,
248 obstetricians require a test that enables a woman to be triaged, to determine those that
249 require increased surveillance, and those where the likelihood of needing delivery for
250 preeclampsia within fourteen days is very low and outpatient care may be appropriate. Such
251 a test would enable development of safe clinical algorithms and avoid inappropriate
252 intervention or unnecessary maternal anxiety.

253 PlGF is an angiogenic factor synthesised by the trophoblast, a marker of associated
254 placental dysfunction in preeclampsia, with known low plasma concentrations in the
255 disease.(16) Whilst combining PlGF with some of the other 46 biologically plausible

biomarkers marginally improved the ROC area, the combinations added little to the diagnostic performance of a single biomarker alone. This important negative result demonstrates the diagnostic option of using a single biomarker (over and above a combination of biomarkers) in preterm preeclampsia. These findings are more marked in women presenting prior to 35 weeks of gestation, and are similar, with lesser diagnostic efficacy, in women presenting between 35⁺⁰ and 36⁺⁶ weeks of gestation. This probably reflects the inclusion of women who meet the primary outcome definition (preeclampsia with delivery within 14 days) who were delivered routinely at 37 weeks of gestation following national guideline recommendations and not because of a clinician concern over a potential placentally-mediated adverse event.

Strengths of this study include use of seven study sites and a large participant cohort, encompassing a wide demographic and ethnic profile including women with underlying maternal disease. Plasma testing was carried out in a central laboratory ensuring that results were obtained with rigorous quality control. Progressive statistical analysis explored single biomarker predictive power, and compared the impact of combining groups of markers, or using biomarker ratios. A limitation was that test results were not validated in a repeat sample or by comparative testing at a second laboratory.

Previous studies have described other pathophysiologically relevant third trimester markers, including soluble endoglin,(17) or measurement of a ratio such as PlGF/ soluble fms-like tyrosine kinase-1.(3, 5) However, some of these studies have been small or from a single centre, often using a case-control design. Such study design can result in over-fitting and does not provide data indicative of how a biomarker may perform if introduced into clinical practice.

279 Systematic reviews have indicated that currently utilised tests such as proteinuria,(8)
280 transaminases(18) and uric acid(19) are not good predictors of maternal or fetal
281 complications in women with suspected preeclampsia. The lack of reliable diagnostic tests
282 results in poorly targeted antenatal monitoring and hospitalisation.(20) Development of an
283 improved diagnostic test, using pathophysiologically relevant biomarkers may have
284 advantages over traditional diagnostic measures.(21) A test performed at presentation that
285 enables targeted surveillance for those at increased risk of maternal or fetal complications
286 and provides appropriate reassurance to those who test negative has the potential to assist
287 in the allocation of health resources.(22) Further work is also needed on prognosis of multi-
288 organ maternal complications in established preeclampsia.

289 Improved detection of placental disease remains a global health priority. Growing
290 evidence suggests the use of angiogenic factors as biomarkers across a range of
291 demographic settings in the prediction of preeclampsia,(4) adverse outcome(23) and
292 placentally related stillbirth.(24) Previous work has shown that women with low or very low
293 PIGF concentrations experienced adverse perinatal outcomes (4) and our findings suggest
294 that increased surveillance should be considered for these women.

295 We have previously reported that PIGF out-performs disease markers currently in use;(4)
296 this study confirms that use of a single angiogenesis-related biomarker may be clinically
297 useful as a diagnostic test, without the need for combinations (which entail additional cost
298 and complexity).. Biomarkers such as PIGF can be analysed quickly, representing a test that
299 could aid risk stratification of women with suspected preterm preeclampsia. Further
300 research, through randomised controlled trials, is essential to assess how these biomarker
301 measurements can assist in determining (or refuting) diagnosis in preeclampsia, and how

this can improve outcomes for mother and baby through optimal tailored clinical management.

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381

382 **Table 1: Characteristics of participants at booking and enrolment for women presenting**
 383 **between 20⁺⁰ and 34⁺⁶ weeks of gestation (according to diagnosis of preeclampsia). Values**
 384 **given are median (quartiles) or n (%) as appropriate.**

Characteristics	Women with preeclampsia requiring delivery within 14 days n=76	All other participants n=210	p value	All women n=286
At booking:				
Age (years)	31.2 (26.8 - 35.6)	32.0 (27.3 - 35.9)	0.84	31.9 (27.0 - 35.8)
Body mass index (kg/m ²)	26.2 (22.8 - 30.1)	29.1 (25.0 - 34.7)	<0.001	28.6 (24.2 - 33.6)
White ethnicity	50 (66)	137 (65)	0.62	187 (65)

Singleton pregnancy	71 (93)	203 (97)	0.27	274 (96)
Highest first trimester systolic BP (mmHg)	120 (110 - 130)	121 (110 - 130)	0.32	120 (110 - 130)
Highest first trimester diastolic BP (mmHg)	70 (65 - 80)	75 (66 - 84)	0.04	74 (66 - 81)
Smoker at booking	11 (15)	42 (21)	0.30	58 (19)
Quit smoking during pregnancy	7 (10)	27 (13)	0.41	34 (12)
Previous medical history:				
Preeclampsia requiring delivery <34 weeks	10 (13)	20 (10)	0.20	30 (11)
Chronic hypertension	7 (10)	38 (19)	0.08	45 (17)
Known SLE or APS	2 (3)	10 (5)	0.44	12 (5)
Pre-existing diabetes mellitus	2 (3)	4 (2)	0.71	6 (2)
Renal disease	5 (7)	14 (7)	0.98	19 (7)
At enrolment:				
Gestational age at sampling (weeks)	32.1 (29.5 - 33.2)	30.9 (26.3 - 33.3)	0.03	31.1 (28.0 - 33.4)
New onset hypertension	53 (70)	101 (48)	<0.001	154 (54)
Worsening of hypertension	14 (18)	42 (20)	0.77	56 (20)
New onset of dipstick	57 (75)	103 (49)	<0.001	160 (56)

proteinuria (1+ or greater)				
Highest systolic BP (mmHg)	150 (140 - 165)	141 (129 - 156)	<0.001	143 (131 - 159)
Highest diastolic BP (mmHg)	97 (88 - 102)	90 (80 - 98)	<0.001	91 (82 - 100)
Alanine transaminase (U/L)	16 (12 - 21)	14 (11 - 19)	0.10	14 (11 - 20)
Creatinine (mg/dl)	0.68 (0.57 – 0.83)	0.55 (0.48 – 0.64)	<0.001	0.58 (0.50 – 0.70)
Uric acid (mg/dl)	5.50 (4.30 - 6.89)	4.03 (3.03 - 4.86)	<0.001	4.32 (3.19 - 5.55)
Platelet count ($\times 10^9/l$)	221 (179 - 269)	238 (204 - 274)	0.06	234 (197 - 271)

385 BP: blood pressure; SLE: systemic lupus erythematosus; APS: antiphospholipid syndrome.

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388 **Table 2: Characteristics of delivery and maternal and neonatal outcome for women**

389 **presenting between 20⁺⁰ and 34⁺⁶ weeks of gestation. Values given are median (quartiles)**

390 **or n (%) as appropriate.**

Characteristics	Women with preeclampsia requiring delivery within 14 days n=76	All other participants n=210	p value	All women n=286
Onset of labour				
Spontaneous	3 (4)	38 (18)	0.01	41 (14)
Induced	13 (17)	95 (45)	<0.001	108 (38)
Pre-labour caesarean section	59 (78)	75 (36)	<0.001	134 (47)
Mode of delivery				
Spontaneous	3 (4)	67 (32)	<0.001	70 (25)
Assisted vaginal delivery	4 (5)	27 (13)	<0.001	31 (11)
Caesarean section	67 (91)	116 (55)	<0.001	183 (64)
Adverse maternal outcome*	37 (49)	84 (40)	0.11	121 (42)
Gestation at delivery (weeks)	32.9 (30 - 34.4)	37.9 (36 - 39.3)	<0.001	36.9 (33.6 - 38.7)
Enrolment to delivery	6.5 (3.0 – 10.0)	43.5 (25.0 –	<0.001	29.5 (11.0 – 59.0)

interval (days)		74.0)		
Neonatal outcomes	n=71	n=203		n=274
Fetal death	3 (4)	3 (2)	0.19	6 (2)
Neonatal death	2 (3)	0 (0)	<0.001	2 (1)
Birthweight (g)	1460 (1030 - 1740)	2900 (2320 - 3350)	<0.001	2500 (1620 - 3170)
Small for gestational age (<10 th birthweight centile)	55 (78)	75 (37)	<0.001	130 (47)
Small for gestational age (<3 rd birthweight centile)	49 (69)	47 (23)	<0.001	96 (35)
Small for gestational age (<1 st birthweight centile)	38 (54)	30 (15)	<0.001	68 (25)
Adverse perinatal outcome†	34 (48)	26 (13)	<0.001	60 (22)

* Adverse maternal outcome defined as presence of any of the following complications:

maternal death, eclampsia, stroke, cortical blindness or retinal detachment, hypertensive

encephalopathy, systolic blood pressure ≥ 160 mmHg, myocardial infarction, Intubation

(other than for caesarean section), pulmonary oedema, platelets $< 50 \times 10^9/L$ (without

transfusion), disseminated intravascular coagulation, thrombotic thrombocytopenic

purpura/ haemolytic uraemic syndrome, hepatic dysfunction (alanine transaminase

≥ 70 IU/L), hepatic haematoma or rupture, acute fatty liver of pregnancy, creatinine > 150

$\mu\text{mol/L}$, renal dialysis, placental abruption, major postpartum haemorrhage, major infection.

399 † Adverse perinatal outcome defined as presence of any of the following complications:
400 antepartum/ intrapartum fetal or neonatal death, neonatal unit admission for >48 hrs at
401 term, intraventricular haemorrhage, periventricular leucomalacia, seizure, retinopathy of
402 prematurity, respiratory distress syndrome, bronchopulmonary dysplasia or necrotising
403 enterocolitis.

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Table 3: ROC areas (95% confidence intervals) for individual biomarkers and combinations (derived from logistic regression) to determine preeclampsia requiring delivery within 14 days of sampling in women presenting for women presenting between 20⁺⁰ and 34⁺⁶ weeks of gestation. [] indicates low concentration of biomarker/ratio correlated to disease.

Biomarkers or combinations	ROC areas (95% confidence intervals)	P value (vs. PlGF alone)
[Pregnancy specific plasma protein A] (PAPP-A)	0.65 (0.57 - 0.72)	<0.001
Procalcitonin	0.65 (0.58 - 0.72)	<0.001
Neutrophil gelatinase-associated lipocalin (NGAL)	0.67 (0.61 - 0.74)	<0.001
Cystatin	0.68 (0.61 - 0.75)	<0.001
Brain natriuretic peptide (BNP)	0.75 (0.69 - 0.82)	<0.001
Interleukin-1 receptor-like 1 (ST2)	0.76 (0.85 - 0.93)	<0.001
Endoglin	0.83 (0.79 - 0.88)	0.08
Soluble fms-like tyrosine kinase-1 (sFlt-1)	0.83 (0.78 - 0.88)	0.07
[Placental growth factor] (PlGF)	0.87 (0.83 - 0.92)	-
Combinations		
[PlGF/sFlt-1 ratio]	0.88 (0.83 - 0.91)	>0.99
[PlGF], Tyrosine kinase (C-Met)	0.88 (0.83 - 0.91)	>0.99
[PlGF/endoglin ratio]	0.88 (0.84 - 0.92)	>0.99
[PlGF], endoglin	0.88 (0.84 - 0.92)	>0.99
[PlGF], ST2	0.89 (0.85 - 0.93)	>0.99

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[PIGF], procalcitonin	0.89 (0.84 - 0.92)	0.86
[PIGF], Cystatin, PAPP-A	0.89 (0.85 - 0.93)	>0.99
[PIGF], Podocalyxin, BNP, procalcitonin	0.90 (0.86 - 0.93)	0.23
[PIGF], Podocalyxin, endoglin, procalcitonin	0.90 (0.86 - 0.93)	0.43

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Table 4: ROC areas (95% confidence intervals) for individual biomarkers and combinations (derived from logistic regression) to determine preeclampsia requiring delivery within 14 days of sampling in women presenting between 35⁺⁰ and 36⁺⁶ weeks of gestation. [] indicates low concentrations of biomarker correlated to disease.

Biomarkers or combinations	ROC areas (95% confidence intervals)	P value (vs. PIGF alone)
Cystatin	0.64 (0.55 - 0.73)	0.11
[Pregnancy specific plasma protein A] (PAPP-A)	0.66 (0.58 - 0.75)	0.12
Neutrophil gelatinase-associated lipocalin (NGAL)	0.67 (0.59 - 0.76)	0.22
Brain natriuretic peptide (BNP)	0.70 (0.61 - 0.78)	0.35
Interleukin-1 receptor-like 1 (ST2)	0.71 (0.63 - 0.79)	0.50
Endoglin	0.71 (0.63 - 0.80)	0.60
Soluble fms-like tyrosine kinase-1 (sFlt-1)	0.75 (0.67 - 0.83)	0.88
[Placental growth factor] (PIGF)	0.75 (0.67 - 0.83)	
Combinations		
[PIGF], procalcitonin	0.73 (0.65 - 0.81)	>0.99
[PIGF], endoglin	0.75 (0.67 - 0.83)	>0.99
[PIGF], Podocalyxin, BNP, procalcitonin	0.76 (0.68 - 0.84)	>0.99
[PIGF], Podocalyxin, sEng, procalcitonin	0.76 (0.68 - 0.83)	>0.99
[PIGF/sFlt-1 ratio]	0.77 (0.69 - 0.84)	>0.99
[PIGF/endoglin ratio]	0.77 (0.66 - 0.82)	>0.99
[PIGF], Cystatin, [PAPP-A]	0.81 (0.74 - 0.88)	0.40

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419 **Figure legends**

420 Figure 1: Participant flow diagram